PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 02 September 1998 (02.09.98)	
International application No. PCT/US98/01394	**Applicant's or agent's file reference** FP64383-RMS
International filing date (day/month/year) 23 January 1998 (23.01.98)	**Priority date** (day/month/year) 24 January 1997 (24.01.97)
Applicant CHAN, Andrew, C. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

18 August 1998 (18.08.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer N. Masson
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
FP64383-RMS		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 98/ 01394	23/01/1998	24/01/1997
Applicant		
WASHINGTON UNIVERSITY et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [X] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. 7
   - [ ] as suggested by the applicant.
   - [X] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/01394

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 A61K38/17 G01N33/58 //C12N15/62

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JACKMAN, J.K. ET AL.: "Molecular cloning of SLP-76, a 76-kDa tyrosine phosphoprotein associated with Grb2 in T cells." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 13, 31 March 1995, pages 7029-7032, XP002064174 cited in the application	1,3, 7-11,17, 19-21
Y	see abstract; figure 2 ---	22
Y	WO 96 30332 A (US HEALTH) 3 October 1996 see page 14, line 31 - line 34; claim 26; examples 8C,9; table 1 ---	22
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
6 May 1998	22/05/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Smalt, R

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FU, C. ET AL.: "Identification of novel Grb2 and PLC-gamma binding proteins in B cell receptor (BCR) function." THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, vol. 99, no. 1, part 2, 1 January 1997, page S469, Abstr. 1905 XP002064175 see abstract	11-15, 17-22
P,X	DATABASE EMBL - EMROD Entry MMAJ814, Acc.No. AJ222814, 12 December 1997 COHEN, P.L. ET AL.: "Mus musculus mRNA for B cell specific protein." XP002064177 see the whole document	1,2,5, 7-14
P,X	FU, C. ET AL.: "Identification of two phosphoproteins, pp70 and pp68, which interact with phospholipase C-gamma, Grb2, and Vav after B cell antigen activation." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 43, 24 October 1997, pages 27362-27368, XP002064176 see the whole document	11-15, 17-22
A	RICHARD S ET AL: "ASSOCIATION OF P62, A MULTIFUNCTIONAL SH2- AND SH3-DOMAIN-BINDING PROTEIN, WITH SRC FAMILY TYROSINE KINASES, GBR2, AND PHOSPHOLIPASE CGAMMY-1" MOLECULAR AND CELLULAR BIOLOGY, vol. 15, no. 1, 1 January 1995, pages 186-197, XP000562643 see the whole document	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/01394

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9630332 A	03-10-96	US 5688992 A	18-11-97
		AU 5377696 A	16-10-96
		CA 2215827 A	03-10-96
		EP 0820433 A	28-01-98

PATENT COOPERATION TREATY

REC'D 03 MAR 1999
WIPO PCT

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
FP64383-RMS		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US98/01394	23/01/1998	24/01/1997
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant WASHINGTON UNIVERSITY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/08/1998	
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. (+49-89) 2399-0 Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer Lonati, M Telephone No. (+49-89) 2399 8160

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US98/01394

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-39 as originally filed

Claims, No.:

1-22 as originally filed

Drawings, sheets:

1/9-9/9 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-16, 18-22
	No:	Claims	17
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-22
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	21-22(?)

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

SECTION V

1. Citations

1.1 The documents mentioned in this IPER are numbered as in the search report, i.e. D1 corresponds to the first document of the search report etc.

1.2 The priority document pertaining to the present application was not available at the time of establishing this IPER. Hence, the current assessment is based on the assumption that all claims enjoy priority rights from the filing date of the priority documents. If it later turns out that this is not correct, documents 4 and 5 cited in the international search report could become relevant to assess whether the present application satisfies the criteria set forth in Article 33(1) PCT.

2. Novelty and Inventive step (Articles 33(2) and 33(3) PCT)

2.1 The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claim 17 is not new in respect of e.g. document D3.

The prior art Grb2, PLC, nck adaptor protein and vav bind to BLNK. Thus, claim 17 is not novel over these molecules.

2.2 The remaining claims 1-16 and 18-22 are considered to be novel as the available documents do not disclose a BLNK protein having the specific sequence depicted in Fig. 1 (SEQ ID NO:1) or homologs which are at least 60% identical to this sequence. However, the clarity objection raised in section VIII, item 1 against the use of arbitrary definitions in the claims should be overcome.

2.3 The claimed subject-matter is not considered to be inventive over the

teaching of D3 for the following reasons.
D3 is a conference abstract from the applicants, disclosing the identification of the 68, 70 and 76 KDa tyrosine-phosphorylated proteins, which interact with PLC-gamma1/2 and Grb2 following BCR stimulation. The 68 K and 70K proteins correspond respectively to the BLNK-2 and the BLNK-1 proteins of the present application.
It is considered that once the proteins have been identified , their molecular cloning by standard procedures of genetic engineering does not involve an inventive sep.
Thus, claims 1-22 are not inventive.

3. **Industrial applicability (Article 33(4) PCT)**

3.1 Claims 1-20 are considered to have industrial applicability.

3.2 For the assessment of present claims 21-22 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.
Claims 21-22 cover also in vivo screening/diagnosis.

SECTION VIII

4. **Clarity (Article 6 PCT)**

4.1 The claimed subject-matter should be defined in technical terms. The arbitrary term "BLNK" used in the claims is not a technical definition of the claimed invention. Thus, the claims should make reference to the specific

sequences reported in Figs 1 and 2.
Owing to the lack of technical characterization of the BLNK protein in claims 1, 3, 7, 16 and 19, documents D1 and D6 could be considered as novelty destroying for claims 1, 3, 7-11, 16, 18-19. Indeed, these documents describe proteins which bind to Grb2 and PLC-gamma1 and could therefore be regarded as functional equivalents of the claimed BLNK protein.

4.2 Claims 7 and 8 should make reference to the DNA of claim 1 and to the host of claim 8, respectively.
Similarly, claims 21 and 22 should make reference to the protein of claim 11.

4.3 Claim 19 seems to have the same scope of claim 18.

WO 98/32852
PCT/US98/013

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

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NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
TRECARTIN, Richard, F.
Flehr, Hohbach, Test, Albritton & Herbert LLP
Suite 3400
4 Embarcadero Center
San Francisco, CA 94111-4187
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 30 July 1998 (30.07.98)	
Applicant's or agent's file reference FP64383-RMS	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/US98/01394	23 January 1998 (23.01.98)	24 January 1997 (24.01.97)

Applicant
WASHINGTON UNIVERSITY et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CA,EP,JP,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   None

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   30 July 1998 (30.07.98) under No. WO 98/32852

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

CORRECTED VERSION*

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/47, A61K 38/17, G01N 33/58 // C12N 15/62

A1

(11) International Publication Number: WO 98/32852

(43) International Publication Date: 30 July 1998 (30.07.98)

(21) International Application Number: PCT/US98/01394

(22) International Filing Date: 23 January 1998 (23.01.98)

(30) Priority Data:

08/788,322	24 January 1997 (24.01.97)	US
08/819,013	17 March 1997 (17.03.97)	US

(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

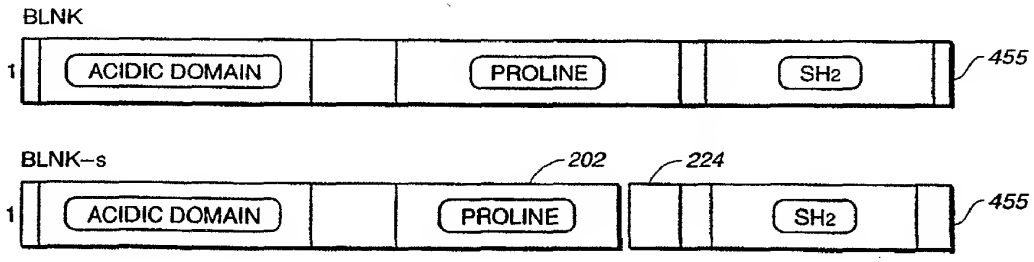
Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(71) Applicant *(for all designated States except US)*: WASHINGTON UNIVERSITY [US/US]; Room 2223, 724 S. Euclid Avenue, St. Louis, MO 63110 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CHAN, Andrew, C. [US/US]; 10962 Chambray Court, St. Louis, MO 63141 (US). FU, Chong [CN/US]; 4570 Clayton Avenue, St. Louis, MO 63110 (US).

(74) Agents: TRECARTIN, Richard, F. et al.; Flehr, Hohbach, Test, Albritton & Herbert LLP, Suite 3400, 4 Embarcadero Center, San Francisco, CA 94111-4187 (US).

(54) Title: BLNK PROTEINS



(57) Abstract

The invention relates to novel BLNK proteins, nucleic acids and antibodies.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
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BLNK PROTEINS

This application is a continuing application of U.S.S.N.s 08/819,013, filed March 17, 1997, and 08/788,322, filed January 24, 1997.

FIELD OF THE INVENTION

The invention relates to novel BLNK proteins, nucleic acids and antibodies.

BACKGROUND OF THE INVENTION

B cell function is dependent upon the ability of the membrane B cell antigen receptor (BCR) to bind antigen and to effectively induce an efficient cascade of biochemical events from the membrane to the nucleus. These events culminate in the cytosol to rearrange the morphology of the cell through cytoskeletal reorganization and in the nucleus to activate the transcription of new genes to promote cellular differentiation and proliferation. These biochemical and cellular events are required for the ability of B cells to mature and function to produce an efficient immune response to foreign pathogens. Conversely, abberant activation of B cells can lead to unregulated cellular proliferation and uncontrolled clonal expansion, resulting in B cell tumors. In addition, unregulated activation of B cells may also contribute to a variety of autoimmune diseases mediated by self-reactive antibodies. Thus, the ability to modulate BCR mediated signaling events may provide a rational approach in the treatment of B-cell mediated tumors and also in autoimmune diseases in which abberant B cell activation may occur.

Ligand binding of the BCR to antigen activates at least two major distinct biochemical pathways within the cell. The first results in increases in intracellular calcium levels that is mediated by an enzyme known as γ isoform of phospholipase C (PLC-γ). Two forms of PLC-γ, γ-1 and γ-2, appear to be capable of mediating this calcium response. The second major biochemical pathway activated through antigen engagement of the BCR is the activation of the ras pathway. Activation of this pathway appears to be mediated by a molecule known as Grb2. Grb2 is an adapter molecule containing two SH3 domains that mediate its interaction with the guanine nucleotide exchange factor, Son of Sevenless (SoS) which in turn activates the ras pathway by facilitating the exchange of GDP for GTP on the ras molecule. Activation of both the ras and calcium pathways are required for efficient BCR function. The molecular mechanisms by which Grb2 and PLC-γ become activated by the BCR remain unclear at this time.

A third, though less characterized pathway, activated by the BCR is cytoskeletal rearrangement and aggregation of the BCR (a phenomenon known as capping). The molecular mechanisms of this pathway are unclear.

While the biochemical mechanisms by which the calcium and ras signaling pathways become activated remain unclear, studies have demonstrated that both of these pathways require the activation at least two families of protein tyrosine kinases (PTKs). The first family is the Src-family of PTKs which can associate with the BCR. A second family is the Syk PTK which interacts with activated, phosphorylated BCR. Abrogation of the function of either Src-PTKs or Syk interferes with calcium and ras signaling pathways and results in a non-functional BCR.

Thus, the discovery of molecules which interact with either Grb2, PLC-γ or Syk, and thus play a role in the regulation of the ras and calcium signaling pathways, are desired. Accordingly, it is an object of the present invention to provide such molecules, termed "BLNK" proteins, and to provide methods of using such molecules in screening assays.

SUMMARY OF THE INVENTION

In accordance with the objects outlined above, the present invention provides recombinant nucleic acids encoding BLNK proteins, and expression vectors and host cells containing the BLNK nucleic acids.

In an additional aspect, the present invention provides methods of producing a BLNK protein comprisingculturing a host cell transformed with nucleic acid encoding a BLNK protein; and expressing the nucleic acid to produce a BLNK protein.

In a further aspect, the present invention provides recombinant BLNK proteins and pharmaceutical compositions comprising BLNK proteins.

In an additional aspect, the present invention provides antibodies which bind BLNK proteins.

In a further aspect, the present invention provides methods for detecting a BLNK protein in a target sample. The methods comprise contacting a labelled polypeptide that binds a BLNK protein with the target sample and assaying for the presence of binding between the labelled polypeptide and BLNK, if present.

In an additional aspect the present invention provides methods for screening for a bioactive agent capable of inhibiting the bioactivity of a BLNK protein. The method comprise combining a BLNK protein and a candidate bioactive agent, and determining the binding of said candidate agent to BLNK protein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the amino acid sequence of human BLNK 1.

Figure 2 depicts the nucleic acid sequence of human BLNK 1.

Figure 3 depicts the amino acid sequence of human BLNK 2.

Figure 4 depicts the nucleic acid sequence of human BLNK 2.

Figure 5 depicts the amino acid sequence of mouse BLNK 1. The human cDNA was used to screen a mouse cDNA library. A full length mouse cDNA encoding BLNK-1 was isolated and sequenced. It demonstrates significant amino acid identity (88%) with human BLNK-1. The mouse only demonstrates a single gene product that corresponds to the full length human BLNK-1.

Figure 6 depicts the nucleic acid sequence of mouse BLNK 1.

Figure 7 depicts a schematic diagram of the BLNK proteins.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel BLNK proteins. In a preferred embodiment, the BLNK proteins are from vertebrates, more preferably from mammals, and in the preferred embodiment, from rats, mice, primates, or humans. However, using the techniques outlined below, BLNK proteins from other organisms may also be obtained.

Without being bound by theory, it appears that there are a number of related BLNK proteins and nucleic acids. For example, in the human, it appears that there are at least two separate BLNK proteins, BLNK 1 and BLNK 2, that result from differential splicing of a single nucleic acid. BLNK-2 is the result of an excision of a 23 amino acid exon from BLNK-1. Both forms are expressed in normal human B cells. In the mouse, there is only one BLNK protein which corresponds to the full length (BLNK 1) protein in humans. These BLNKs share basic homology to each other, and a general lack of homology to any other known proteins or nucleic acids.

Thus, a BLNK protein of the present invention may be identified in several ways. A BLNK nucleic acid or BLNK protein is initially identified by substantial nucleic acid and/or amino acid sequence homology to the sequences shown in Figures 1, 2, 3, 4, 5 or 6. Such homology can be based upon the overall nucleic acid or amino acid sequence.

As used herein, a protein is a "BLNK protein" if the overall homology of the protein sequence to the amino acid sequences shown in Figures 1, 3 or 5 is preferably greater than about 40%, more preferably greater than about 50% and most preferably greater than 75-80%. In some embodiments the homology will be as high as about 90 to 95 or 98%. This homology will be determined using standard techniques known in the art, such as the Best Fit sequence program described by Devereux *et al.*, *Nucl. Acid Res. 12*:387-395 (1984) or the BLASTX program (Altschul et al., J. Mol. Biol. *215*, 403-410). The alignment may include the introduction of gaps in the sequences to be aligned. In addition, for sequences which contain either more or fewer amino acids than the proteins shown in the Figures, it is understood that the percentage of homology will be determined based on the number of homologous amino acids in relation to the total number of amino acids. Thus, for example, homology of sequences shorter than that shown in the Figures, as discussed below, will be determined using the number of amino acids in the shorter sequence.

BLNK-1 has limited homology to SLP-76, an SH2 containing leukcyte protein of 76,000 MW. SLP-76 is a protein that undergoes tyrosine phosphorylation following activation of the T cell antigen receptor (TCR). SLP-76 was cloned (see Jackman et al., J. Biol. Chem. 270:7029-7032 (1995)) as a tyrosine phosphorylated molecule that interacts with Grb2 and PLC-γ-1. Similar to BLNK-1, SLP-76 also has the three structural regions depicted in Figure 7. The tyrosine phosphorylation of SLP-76 is required for TCR mediated cytokine secretion. The overall homology between SLP-76 and BLNK-1 is 33% identity.

As discussed above, BLNK proteins include BLNK 1 and BLNK 2 proteins. BLNK proteins may be identified in one aspect by significant homology to at least one and preferably all of the regions schematically shown in Figure 7, including the acidic region (residues 51-109),

the proline rich region in the central portion of the molecule (130-345), putatively identified as a binding site for SH-3 containing proteins, and an SH2 domain at the C-terminus (346-438).

The N-terminal region of BLNK protein contains an acidic region (amino acids 51-109). The amino acid consensus sequence of this area is rather loose, and consists basically of three YEXP sequences preseceded by a negatively charged amino acid (D or E) in the -2 position. The consensus sequence is D/EXYEXPX D/EXYXXPXD/EX YEPP.

This acidic region contains tyrosine phosphorylation sites at (human BLNK-1 numbering) Tyr 71, Tyr83, Tyr95, Tyr177, and Tyr187. Biochemical and genetic approaches have confirmed that all of these tyrosines are phosphorylated following B-cell activation, and that it is the Syk protein tyrosine kinase that is responsible for phosphorylating BLNK following B cell activation. Syk can phosphorylate BLNK when co-expressed in insect cells, and in vitro. BLNK is not phosphorylated in cells lacking Syk.

The central portion of BLNK proteins contain a proline rich region, amino acids 130-345, which serves as the binding site for SH3 containing proteins. This site has been implicated as the binding site for Grb2.

The C-terminus of BLNK proteins contains an SH2 domain, amino acids 346-438, which may bind tyrosine phosphorylated proteins. SH2 domains are known to be involved in binding of signal pathway proteins.

BLNK proteins of the present invention may be shorter or longer than the amino acid sequences shown in the Figures. Thus, in a preferred embodiment, included within the definition of BLNK proteins are portions or fragments of the sequences shown in Figures 1, 2, 3, 4, 5 and 6. Alternatively, longer fragments of BLNK proteins can be made.

BLNK proteins may also be identified as being encoded by BLNK nucleic acids. Thus, BLNK proteins are encoded by nucleic acids that will hybridize to the sequences depicted in Figures 2, 4 and 6, as outlined herein. In a preferred embodiment, high stringency conditions are used, as are known in the art.

In a preferred embodiment, when the BLNK protein is to be used to generate antibodies, the BLNK protein must share at least one epitope or determinant with the full length protein. By "epitope" or "determinant" herein is meant a portion of a protein which will generate and/or bind an antibody. Thus, in most instances, antibodies made to a smaller BLNK protein will be able to bind to the full length protein. In a preferred embodiment, the epitope is unique; that is, antibodies generated to a unique epitope show little or no cross-reactivity. The BLNK antibodies of the invention specifically bind to BLNK proteins. By "specifically bind" herein is meant that the antibodies bind to the protein with a binding constant in the range of at least 10^4- 10^6 M^{-1}, with a preferred range being 10^7 - 10^9 M^{-1}. The antibodies may be either polyclonal or monoclonal, with monoclonal antibodies being preferred.

In the case of the nucleic acid, the overall homology of the nucleic acid sequence is commensurate with amino acid homology but takes into account the degeneracy in the genetic code and codon bias of different organisms. Accordingly, the nucleic acid sequence homology may be either lower or higher than that of the protein sequence. Thus the homology of the nucleic acid sequence as compared to the nucleic acid sequence of Figures 2, 4 or 6 is preferably greater than 40%, more preferably greater than about 45%, particularly greater than about 50% and most preferably greater than 55%. In some embodiments the homology will be as high as about 70, 80, 90, 95 or 98%.

In a preferred embodiment, a BLNK nucleic acid encodes a BLNK protein; BLNK 1 nucleic acids encode BLNK 1 proteins, and BLNK 2 nucleic acids encode BLNK 2 proteins. As will be appreciated by those in the art, due to the degeneracy of the genetic code, an extremely large number of nucleic acids may be made, all of which encode the BLNK proteins of the present invention. Thus, having identified a particular amino acid sequence, those skilled in

the art could make any number of different nucleic acids, by simply modifying the sequence of one or more codons in a way which does not change the amino acid sequence of the BLNK.

In one embodiment, the nucleic acid homology is determined through hybridization studies. Thus, for example, nucleic acids which hybridize under high stringency to the nucleic acid sequences shown in Figures 2, 4 or 6 or their complements are considered BLNK genes. High stringency conditions are known in the art; see for example Maniatis et al., Molecular Cloning: A Laboratory Manual, 2d Edition, 1989, and Short Protocols in Molecular Biology, ed. Ausubel, et al., both of which are hereby incorporated by reference.

In another embodiment, less stringent hybridization conditions are used; for example, moderate or low stringency conditions may be used, as are known in the art; see Maniatis and Ausubel, supra.

The BLNK proteins and nucleic acids of the present invention are preferably recombinant. As used herein, "nucleic acid" may refer to either DNA or RNA, or molecules which contain both deoxy- and ribonucleotides. The nucleic acids include genomic DNA, cDNA and oligonucleotides including sense and anti-sense nucleic acids. Such nucleic acids may also contain modifications in the ribose-phosphate backbone to increase stability and half life of such molecules in physiological environments.

The nucleic acid may be double stranded, single stranded, or contain portions of both double stranded or single stranded sequence. By the term "recombinant nucleic acid" herein is meant nucleic acid, originally formed *in vitro*, in general, by the manipulation of nucleic acid by endonucleases, in a form not normally found in nature. Thus an isolated BLNK nucleic acid, in a linear form, or an expression vector formed *in vitro* by ligating DNA molecules that are not normally joined, are both considered recombinant for the purposes of this invention. It is understood that once a recombinant nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, i.e. using the in vivo cellular machinery of the

host cell rather than *in vitro* manipulations; however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purposes of the invention.

Similarly, a "recombinant protein" is a protein made using recombinant techniques, i.e. through the expression of a recombinant nucleic acid as depicted above. A recombinant protein is distinguished from naturally occurring protein by at least one or more characteristics. For example, the protein may be isolated or purified away from some or all of the proteins and compounds with which it is normally associated in its wild type host, and thus may be substantially pure. For example, an isolated protein is unaccompanied by at least some of the material with which it is normally associated in its natural state, preferably constituting at least about 0.5%, more preferably at least about 5% by weight of the total protein in a given sample. A substantially pure protein comprises at least about 75% by weight of the total protein, with at least about 80% being preferred, and at least about 90% being particularly preferred. The definition includes the production of a BLNK protein from one organism in a different organism or host cell. Alternatively, the protein may be made at a significantly higher concentration than is normally seen, through the use of a inducible promoter or high expression promoter, such that the protein is made at increased concentration levels. Alternatively, the protein may be in a form not normally found in nature, as in the addition of an epitope tag or amino acid substitutions, insertions and deletions, as discussed below.

Also included with the definition of BLNK protein are other BLNK proteins of the BLNK family, and BLNK proteins from other organisms, which are cloned and expressed as outlined below. Thus, probe or degenerate polymerase chain reaction (PCR) primer sequences may be used to find other related BLNK proteins from humans or other organisms. As will be appreciated by those in the art, particularly useful probe and/or PCR primer sequences include the unique areas of the BLNK nucleic acid sequence. Thus, useful probe or primer sequences may be designed to all or part of the acidic region. As is generally known in the art, preferred PCR primers are from about 15 to about 35 nucleotides in length,

with from about 20 to about 30 being preferred, and may contain inosine as needed. The conditions for the PCR reaction are well known in the art.

Once the BLNK nucleic acid is identified, it can be cloned and, if necessary, its constituent parts recombined to form the entire BLNK protein nucleic acid. Once isolated from its natural source, e.g., contained within a plasmid or other vector or excised therefrom as a linear nucleic acid segment, the recombinant BLNK nucleic acid can be further used as a probe to identify and isolate other BLNK nucleic acids. It can also be used as a "precursor" nucleic acid to make modified or variant BLNK nucleic acids and proteins.

Using the nucleic acids of the present invention which encode a BLNK protein, a variety of expression vectors are made. The expression vectors may be either self-replicating extrachromosomal vectors or vectors which integrate into a host genome. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleic acid encoding the BLNK protein. "Operably linked" in this context means that the transcriptional and translational regulatory DNA is positioned relative to the coding sequence of the BLNK protein in such a manner that transcription is initiated. Generally, this will mean that the promoter and transcriptional initiation or start sequences are positioned 5' to the BLNK protein coding region. The transcriptional and translational regulatory nucleic acid will generally be appropriate to the host cell used to express the BLNK protein; for example, transcriptional and translational regulatory nucleic acid sequences from *Bacillus* are preferably used to express the BLNK protein in *Bacillus*. Numerous types of appropriate expression vectors, and suitable regulatory sequences are known in the art for a variety of host cells.

In general, the transcriptional and translational regulatory sequences may include, but are not limited to, promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. In a preferred embodiment, the regulatory sequences include a promoter and transcriptional start and stop sequences.

Promoter sequences encode either constitutive or inducible promoters. The promoters may be either naturally occurring promoters or hybrid promoters. Hybrid promoters, which combine elements of more than one promoter, are also known in the art, and are useful in the present invention.

In addition, the expression vector may comprise additional elements. For example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in mammalian or insect cells for expression and in a procaryotic host for cloning and amplification. Furthermore, for integrating expression vectors, the expression vector contains at least one sequence homologous to the host cell genome, and preferably two homologous sequences which flank the expression construct. The integrating vector may be directed to a specific locus in the host cell by selecting the appropriate homologous sequence for inclusion in the vector. Constructs for integrating vectors are well known in the art.

In addition, in a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

The BLNK proteins of the present invention are produced by culturing a host cell transformed with an expression vector containing nucleic acid encoding a BLNK protein, under the appropriate conditions to induce or cause expression of the BLNK protein. The conditions appropriate for BLNK protein expression will vary with the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the art through routine experimentation. For example, the use of constitutive promoters in the expression vector will require optimizing the growth and proliferation of the host cell, while the use of an inducible promoter requires the appropriate growth conditions for induction. In addition, in some embodiments, the timing of the harvest is important. For example, the baculoviral systems used in insect cell expression are lytic viruses, and thus harvest time selection can be crucial for product yield.

Appropriate host cells include yeast, bacteria, archebacteria, fungi, and insect and animal cells, including mammalian cells. Of particular interest are *Drosophila melangaster* cells, *Saccharomyces cerevisiae* and other yeasts, *E. coli*, *Bacillus subtilis*, SF9 cells, C129 cells, 293 cells, Neurospora, BHK, CHO, COS, and HeLa cells, fibroblasts, Schwanoma cell lines, and immortalized mammalian myeloid, lymphoid cell lines, and insect Hi5 cells.

In a preferred embodiment, the BLNK proteins are expressed in mammalian cells. Mammalian expression systems are also known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence for BLNK protein into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, using a located 25-30 base pairs upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element (enhancer element), typically located within 100 to 200 base pairs upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation. Of particular use as mammalian promoters are the promoters from mammalian viral genes, since the viral genes are often highly expressed and have a broad host range. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter, herpes simplex virus promoter, and the CMV promoter.

Typically, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-translational cleavage and polyadenylation. Examples of transcription terminator and polyadenlytion signals include those derived form SV40.

The methods of introducing exogenous nucleic acid into mammalian hosts, as well as other hosts, is well known in the art, and will vary with the host cell used. Techniques include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, direct microinjection of the DNA into nuclei, etc.

In a preferred embodiment, BLNK proteins are expressed in bacterial systems. Bacterial expression systems are well known in the art.

A suitable bacterial promoter is any nucleic acid sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of the coding sequence of BLNK protein into mRNA. A bacterial promoter has a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region typically includes an RNA polymerase binding site and a transcription initiation site. Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose and maltose, and sequences derived from biosynthetic enzymes such as tryptophan. Promoters from bacteriophage may also be used and are known in the art. In addition, synthetic promoters and hybrid promoters are also useful; for example, the *tac* promoter is a hybrid of the *trp* and *lac* promoter sequences. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription.

In addition to a functioning promoter sequence, an efficient ribosome binding site is desirable. In *E. coli*, the ribosome binding site is called the Shine-Delgarno (SD) sequence and includes an initiation codon and a sequence 3-9 nucleotides in length located 3 - 11 nucleotides upstream of the initiation codon.

The expression vector may also include a signal peptide sequence that provides for secretion of the BLNK protein in bacteria. The signal sequence typically encodes a signal peptide

comprised of hydrophobic amino acids which direct the secretion of the protein from the cell, as is well known in the art. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria).

The bacterial expression vector may also include a selectable marker gene to allow for the selection of bacterial strains that have been transformed. Suitable selection genes include genes which render the bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin, neomycin and tetracycline. Selectable markers also include biosynthetic genes, such as those in the histidine, tryptophan and leucine biosynthetic pathways.

These components are assembled into expression vectors. Expression vectors for bacteria are well known in the art, and include vectors for *Bacillus subtilis, E. coli, Streptococcus cremoris*, and *Streptococcus lividans*, among others.

The bacterial expression vectors are transformed into bacterial host cells using techniques well known in the art, such as calcium chloride treatment, electroporation, and others.

In one embodiment, BLNK proteins are produced in insect cells. Expression vectors for the transformation of insect cells, and in particular, baculovirus-based expression vectors, are well known in the art.

In a preferred embodiment, BLNK protein is produced in yeast cells. Yeast expression systems are well known in the art, and include expression vectors for *Saccharomyces cerevisiae*, *Candida albicans* and *C. maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis* and *K. lactis*, *Pichia guillerimondii* and *P. pastoris*, *Schizosaccharomyces pombe*, and *Yarrowia lipolytica*. Preferred promoter sequences for expression in yeast include the inducible GAL1,10 promoter, the promoters from alcohol dehydrogenase, enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase,

hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, pyruvate kinase, and the acid phosphatase gene. Yeast selectable markers include ADE2, HIS4, LEU2, TRP1, and ALG7, which confers resistance to tunicamycin; the neomycin phosphotransferase gene, which confers resistance to G418; and the CUP1 gene, which allows yeast to grow in the presence of copper ions.

The BLNK protein may also be made as a fusion protein, using techniques well known in the art. Thus, for example, for the creation of monoclonal antibodies, if the desired epitope is small, the BLNK protein may be fused to a carrier protein to form an immunogen. For example, two fusion proteins have been generated to make antibodies; amino acids 4-205 and amino acids 324-457 of human BLNK-1 have each been fused to glutathione-S-transferase, the fusion proteins produced in bacteria and used as immunogens in rabbits (polyclonal antibodies) and mice (monoclonal antibodies). The rabbit polyclonal antibodies immunoprecipitate and immunoblot both human and mouse BLNK-1 (data not shown), and the mouse monoclonal antibodies immunoprecipitate and immunoblot only human BLNK-1 (data not shown). Alternatively, the BLNK protein may be made as a fusion protein to increase expression, or to include an epitope tag or purification tag (i.e. His_6) to allow purification, or for other reasons.

Also included within the definition of BLNK proteins of the present invention are amino acid sequence variants. These variants fall into one or more of three classes: substitutional, insertional or deletional variants. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the BLNK protein, using cassette or PCR mutagenesis or other techniques well known in the art, to produce DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture as outlined above. However, variant BLNK protein fragments having up to about 100-150 residues may be prepared by *in vitro* synthesis using established techniques. Amino acid sequence variants are characterized by the predetermined nature of the variation, a feature that sets them apart from naturally occurring allelic or interspecies variation of the BLNK protein amino acid sequence. The variants typically exhibit the same qualitative biological activity as the naturally occurring

analogue, although variants can also be selected which have modified characteristics as will be more fully outlined below.

While the site or region for introducing an amino acid sequence variation is predetermined, the mutation per se need not be predetermined. For example, in order to optimize the performance of a mutation at a given site, random mutagenesis may be conducted at the target codon or region and the expressed BLNK variants screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example, M13 primer mutagenesis and PCR mutagenesis. Screening of the mutants is done using assays of BLNK protein activities; for example, for binding domain mutations, competitive binding studies such as are outlined in the Examples may be done.

Amino acid substitutions are typically of single residues; insertions usually will be on the order of from about 1 to 20 amino acids, although considerably larger insertions may be tolerated. Deletions range from about 1 to about 20 residues, although in some cases deletions may be much larger. For example, preferred deletion variants include the deletion of one or more of the three domains, i.e. the acidic domain, the proline rich region, or the SH2 domain.

Substitutions, deletions, insertions or any combination thereof may be used to arrive at a final derivative. Generally these changes are done on a few amino acids to minimize the alteration of the molecule. However, larger changes may be tolerated in certain circumstances. When small alterations in the characteristics of the BLNK protein are desired, substitutions are generally made in accordance with the following chart:

Chart I

Original Residue	Exemplary Substitutions
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile
Phe	Met, Leu, Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu

Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those shown in Chart I. For example, substitutions may be made which more significantly affect: the structure of the polypeptide backbone in the area of the alteration, for example the alpha-helical or beta-sheet structure; the charge or hydrophobicity of the molecule at the target site; or the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the polypeptide's properties are those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g. lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g. glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g. phenylalanine, is substituted for (or by) one not having a side chain, e.g. glycine.

The variants typically exhibit the same qualitative biological activity and will elicit the same immune response as the naturally-occurring analogue, although variants also are selected to modify the characteristics of the BLNK proteins as needed. Alternatively, the variant may be designed such that the biological activity of the BLNK protein is altered. For example, any or all of the three domains may be altered, i.e. the acidic domain, the proline rich region, or the SH2 domain. For example, one or more of the tyrosine phosphorylation sites may be altered; as outlined herein, a mutant human BLNK-1 has been made which replaces all five of the tyrosine phosphorylation sites with phenylalanine has been done.

In one embodiment, the BLNK nucleic acids, proteins and antibodies of the invention are labelled. By "labelled" herein is meant that a compound has at least one element, isotope or chemical compound attached to enable the detection of the compound. In general, labels fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes; b) immune labels, which may be antibodies or antigens; and c) colored or fluorescent dyes. The labels may be incorporated into the compound at any position.

In a preferred embodiment, the BLNK protein is purified or isolated after expression. BLNK proteins may be isolated or purified in a variety of ways known to those skilled in the art depending on what other components are present in the sample. Standard purification methods include electrophoretic, molecular, immunological and chromatographic techniques, including ion exchange, hydrophobic, affinity, and reverse-phase HPLC chromatography, and chromatofocusing. For example, the BLNK protein may be purified using a standard anti-BLNK antibody column. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. For general guidance in suitable purification techniques, see Scopes, R., Protein Purification, Springer-Verlag, NY (1982). The degree of purification necessary will vary depending on the use of the BLNK protein. In some instances no purification will be necessary.

Once expressed and purified if necessary, the BLNK proteins are useful in a number of applications.

BLNK appears to be involved in B cell function in several ways. BLNK is able to interact with phospholipase C, an enzyme that can hydrolyze and activate inositol phospholipids to increase free cytoplasmic calcium levels. Overexpression of wild-type BLNK can increase tyrosine phosphorylation of BLNK and result in additional increases in free cytoplasmic calcium levels. Conversely, expression of a mutant BLNK in which the tyrosine residues that are phosphorylated are substituted with phenylalanine (and thus are unable to be phosphorylated) will decrease calcium levels following B cell activation. Accordingly, one of BLNK's functions is to modulate the ability of the B cell receptor to regulate calcium levels in the cell.

In addition, BLNK has been shown to interact with a variety of proteins in B cells. In addition to Grb2 and PLC, BLNK also interacts with the nck adaptor protein and the Vav protooncogene following BCR activation. Preliminary data (not shown) suggests that these two interactions likely control the ability of B cells to regulate actin polymerization and cytoskeletal reorganization. The cytoskeleton has been demonstrated in other systems to be important in B cell function and in tumor invasiveness, and may serve as a therapeutic target through BLNK to treat B cell related tumors.

As outlined in the examples, Northern blot analysis of BLNK-1 demonstrates that BLNK-1 demonstrates its highest level of expression in spleen/B cells and substantially lower levels of expression, if any, in all other tissues examined. Thus, BLNK-1 has a relatively specific pattern of expression, and thus is highly specific for B-cells. This makes it useful in a number of ways, as there are not many specific B cell markers.

In a preferred embodiment, BLNK proteins and nucleic acids are used in screening assays of candidate bioactive agents that modulate BLNK bioactivity, for potential use to treat B-cell lymphomas. By "modulate" herein is meant that the bioactivity of BLNK is altered, either increased or decreased. In a preferred embodiment, the bioactivity is inhibited. BLNK is putatively critical for the BCR response, and thus for B cell function. Accordingly, BLNK may be used as a target to screen for inhibitors of its function or expression.

In a preferred embodiment, BLNK proteins and nucleic acids are used in screening assays of candidate bioactive agents that modulate BLNK bioactivity, for potential use to treat autoimmune diseases which have hyperactivated B cells.

Thus, in a preferred embodiment, the methods comprise screening for a bioactive agent capable of inhibiting the bioactivity of a BLNK protein. By "bioactivity" herein is meant the binding of the BLNK to any of its targets, including Grb2, PLC-γ, the nck adaptor protein and the VAV protooncogene, the regulation of calcium levels, or cytoskeletal organization. That is, bioactive agents that prevent BLNK binding, i.e. interrupt the interaction of BLNK and its target, may be found. The method comprises combining a BLNK protein and a candidate bioactive agent, and determining the binding of the candidate agent to BLNK protein.

Generally, in a preferred embodiment of the methods herein, a BLNK polypeptide is non-diffusably bound to an insoluble support having isolated sample receiving areas (e.g. a microtiter plate, an array, etc.). The insoluble supports may be made of any composition to which polypeptides can be bound, is readily separated from soluble material, and is otherwise compatible with the overall method of screening. The surface of such supports may be solid or porous and of any convenient shape. Examples of suitable insoluble supports include microtiter plates, arrays, membranes and beads. These are typically made of glass, plastic (e.g., polystyrene), polysaccharides, nylon or nitrocellulose. Microtiter plates and arrays are especially convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples. The particular manner of binding of the polypeptide is not crucial so long as it is compatible with the reagents and overall methods of the invention, maintains the activity of the peptide and is nondiffusable. Preferred methods of binding include the use of antibodies (which should not hinder the binding of BLNK to its targets, including Grb2, PLC-γ, nck or Vav), direct binding to "sticky" or ionic supports, chemical crosslinking, etc. Following binding of the polypeptide, excess unbound material is removed by washing. The sample receiving areas may then be blocked as needed through incubation with bovine serum albumin (BSA), casein or other innocuous protein.

A candidate bioactive agent is added to the assay. Novel binding agents include specific antibodies, non-natural binding agents identified in screens of chemical libraries, peptide analogs, etc. Of particular interest are screening assays for agents that have a low toxicity for human cells. A wide variety of assays may be used for this purpose, including labeled in vitro protein-protein binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, and the like.

The term "agent" as used herein describes any molecule, e.g., protein, oligopeptide, small organic molecule, polysaccharide, polynucleotide, etc., with the capability of directly or indirectly altering the bioactivity of BLNK proteins. Generally a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration or below the level of detection.

Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 100 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally,

natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification to produce structural analogs.

The determination of the binding of the candidate bioactive agent to the BLNK polypeptide may be done in a number of ways. In one embodiment, the candidate bioactive agent is labelled, and binding determined directly.

Where the screening assay is a binding assay, one or more of the molecules may be joined to a label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescers, chemiluminescers, specific binding molecules, particles, e.g. magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin etc. For the specific binding members, the complementary member would normally be labeled with a molecule which provides for detection, in accordance with known procedures. In some embodiments, only one of the components is labeled. Alternatively, more than one component may be labeled with different labels; for example, the BLNK polypeptide may be labeled with one fluorophor and the candidate agent labeled with another.

In one embodiment, the candidate bioactive agent is labeled. The labeled candidate bioactive agents are added to the BLNK polypeptide for a time sufficient to allow binding, if present. Incubations may be performed at any temperature which facilitates optimal activity, typically between 4 and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high through put screening. Typically between 0.1 and 1 hour will be sufficient. Excess reagent is generally removed or washed away. The presence or absence of the labeled component is followed, to indicate binding.

In a preferred embodiment, the methods comprise combining a BLNK protein, a candidate bioactive agent, and either Grb2 or PLC-γ, and determining the binding of the BLNK to one

of its targets, including Grb2, PLC-γ, nck or Vav, to determine the effect of the candidate bioactive agent.

Thus, in a preferred embodiment, the methods comprise differential screening to identity bioactive agents that are capable of modulating the bioactivity of BLNK proteins. In this embodiment, the methods comprise combining a BLNK polypeptide and one of BLNK's binding partners, including Grb2, PLC-γ, nck, or Vav, in a first sample. A second sample comprises a candidate bioactive agent, BLNK polypeptide and one of BLNK's binding partners. The binding of BLNK to its binding partner is determined for both samples, and a change, or difference in binding between the two samples indicates the presence of an agent capable of modulating the bioactivity of BLNK.

Alternatively, a preferred embodiment utilizes differential screening to identify drug candidates that bind to the native BLNK, but cannot bind to modified BLNK proteins, for example those that have modifications which eliminate or decrease bioactivity of a BLNK protein; for example BLNK proteins that may no longer be phosphorylated, as outlined herein.

Preferably all control and test samples are performed in at least triplicate to obtain statistically significant results. Incubation of all samples is for a time sufficient for the binding of the BLNK proteins and the Grb2 and/or PLC-γ proteins. Following incubation, all samples are washed free of non-specifically bound material and the amount of bound, labeled material determined. For example, where a radiolabel is employed as a label, the samples may be counted in a scintillation counter to determine the amount of labeled compound.

A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, e.g. albumin, detergents, etc which may be used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used.

The mixture of components may be added in any order that provides for the requisite binding.

The following examples serve to more fully describe the manner of using the above-described invention, as well as to set forth the best modes contemplated for carrying out various aspects of the invention. It is understood that these examples in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes. All references cited herein are incorporated by reference.

EXAMPLES

Cloning and Characterization of BLNK proteins

Using B cell tumor cell lines and normal B cells, two proteins with apparent molecular masses of 68,000, 70,000, and 76,000 that interact with Grb2 and PLCγ1 following BCR activation were observed. Moreover, these proteins became phosphorylated on tyrosine residues following BCR crosslinking. Using immunodepletion studies, we demonstrated that the 68K and 70K Mr proteins that interacted with Grb2 and PLCγ1 were in fact the same protein. More recent data has also suggested that the 68K and 70 K Mr proteins also interact with nck following BCR activation. These initial data suggested to us that these proteins may serve as important regulatory proteins to link the BCR and its associated PTKs with these major downstream signaling functions. Given the ability of these proteins to likely link the proximal BCR and its associated PTKs with downstream signaling functions, we have designated the 68,000 Mr protein as BLNK-2 (B cell LINKER PROTEIN) and the 70,000 Mr protein as BLNK-1.

To further characterize these proteins, we undertook a large scale purification scheme of the 68K and 70K Mr proteins from human B cells. We have derived peptide sequences for both of these proteins and have identified a full length cDNA for human BLNK-1 (the 70K Mr protein). The predicted open reading frame of the BLNK-1 cDNA was consistent with the peptides derived from protein sequencing. This novel cDNA is not represented in the GenBank data base (December, 1996). Northern blot analysis of BLNK-1 demonstrates its

highest level of expression in spleen/B cells and substantially lower levels of expression, if any, in all other tissues examined. Thus, BLNK-1 has a relatively specific pattern of expression for B cells.

Analysis of PCR products using cDNA from the human Ramos B cell line demonstrated the presence of two gene products. DNA sequencing of these two gene products demonstrated that these two gene products differ by the absence of 23 amino acids from the BLNK-1 open reading frame. Hence, BLNK-2 appears to be an alternatively spliced form of BLNK-1; a 23 amino acid exon of BLNK-1 is excised in BLNK-2. Thus, it is likely that BLNK-1 and 2 form a family of proteins that serve to link the BCR with a variety of signaling and adapter proteins (e.g., Grb2, nck, VAV and PLCg1) to mediate B cell activation.

The open reading frame of BLNK-1 contained three intriguing structural regions (Figure 7). The amino (N)-terminus contains an acidic region which has three potential tyrosine residues for phosphorylation. The central portion of the molecule has a proline rich region which may serve as the binding site for SH3 containing proteins. Binding studies suggest that this region likely serves as the binding site for Grb2. Finally, the carboxy-terminal portion of the molecule has an SH2 domain for binding other tyrosine phosphorylated proteins. Homology search of the protein sequence with the GenBank database revealed homology (33% amino acid identity) with the protein, SLP-76 (SH2-containing leukocyte protein of 76,000 Mr).

Protein Purification and Microsequencing: Ramos Burkit Lymphoma cells (ATCC) were cultured in RPMI-1640 with 10% fetal calf serum. 1.5 x 1010 cells were collected by centrifugation (1000g x 10 min) and washed once with ice-cold phosphate buffered saline (PBS). The cells were then resuspended in 75 ml ice-cold PBS and divided into 50 microcentrifuge tubes (1.5 ml each). 25 ml anti-hIgM (1.2 mg/ml, Jackson Labs) were added to each tube and inverted twice. Cells were incubated with the stimulating antibody for 5-30 minutes on ice, sedimented by flash centrifugation, and resuspended in an equal volume of pre-warmed PBS (37°C) for 1.5 min. Following stimulation, cells were sedimented and lysed in equal volume of lysis buffer (1% NP-40, 10mM Tris-HCl, pH 8.0, 150 mM NaCl) with

protease and phosphatase inhibitors (10 mM NaF, 1 mM sodium orthovanadate, 10 mg/ml phenylmethanesulfonyl- fluoride, 2 mg/ml aprotinin, 1 mg/ml leupeptin, 2 mg/ml pepstatin, 5 mM EDTA, 10 mg/ml soybean trypsin inhibitor). Cell lysates were pooled and cleared by centrifugation (15,000g x 20 min) and the supernatants were further cleared by ultra-centrifugation (100,000g x 40 min). Cleared lysates were passed through a 2 ml column of glutathione S-transferase (GST) immobilized on Glutathione-Sepharose (Pharmacia, 2 mg/ml). The flowthrough from the GST-Sepharose column was then incubated for ~12 hrs with 2 ml of a 50% slurry of a fusion protein consisting of GST and the C-terminal SH2 domain of PLCg1 [designated as GST-PLCg1(C-SH2)] immobilized on Glutathione-Sepharose. Following binding, the GST-PLCg1(C-SH2) beads were transferred to a column, washed twice with 15 ml high salt lysis buffer (lysis buffer with 0.5 mM NaCl), and twice with 15 ml lysis buffer. Beads were transferred to a 15 ml conical tube and washed with 15 ml lysis buffer. Proteins bound to GST-PLCg1(C-SH2) were then eluted twice by incubating the beads with 2 ml lysis buffer containing 1% SDS for 5 min at 95oC and eluants combined (4 ml). Lysis buffer was added to dilute the SDS to 0.1%. The diluted sample was incubated with 1 ml 50% slurry of an anti-phosphotyrosine antibody (PY20) immobilized on Protein A-Sepharose (1 mg/ml) for 5 hrs at 4°C. The beads were then washed with lysis buffer (5 x 10 ml), transferred to 2 microcentrifuge tubes, washed two additional times with low salt buffer A (20 mM Tris-HCl, pH 8.0, 25 mM NaCl), and eluted twice with 500 ml/tube of 100 mM phenylphosphate (Sigma) in buffer A. The eluants were combined (2 ml), adjusted to 0.1% SDS, and dialyzed against buffer A containing 0.1% SDS.

Three similar scale preparations (1.5×10^{10} cells each) were combined and the final sample (6 ml) was concentrated using a Centriplus Concentrator (Amicon). Proteins were resolved on a 1.5 mm 7% SDS-polyacrylamide gel and visualized with Coommasie Brilliant Blue R 250 (Biorad). Proteins of interest were excised and subjected to enzymatic digestion. Peptides were resolved by standard biochemical techniques using HPLC and sequenced using automated Edman degradation.

Immobilization of GST-fusion proteins: GST fusion proteins were incubated with Glutathione Sepharose (Pharmacia) in PBS for 30 min at 4°C, washed extensively with PBS, and subsequently incubated at room temperature for 30 minutes with 10 bead-volumes of freshly prepared dimethylpimelimidate (Sigma) in 50mM carbonate buffer, pH 9.2. The beads were then washed once with 0.2 M ethnolamine (pH 8.0) and rotated for 2 hours with 10 bead-volumes of 0.2 M ethanolamine (pH 8.0). Beads were again washed with PBS and stored at 4°C in PBS, 0.1% sodium azide. Immobilization of PY20 on Protein A-Sepharose (Pharmacia) were performed in a similar fashion.

cDNA cloning of BLNK-1: Degenerate oligonucleotides based on peptide sequences were used (TCGAGAATTCAAA/GAAA/GCCIACNACNCC from the peptide KKPTTPLK and CTGAGGATCCTTIGTNGCC/TTCG/A/TATA/GAA from KRVYNIPVRFIEATK). Ramos mRNA was used for first strand cDNA synthesis using random primers and the degenerate oligonucleotides used as the 5' and 3' primers. Conditions used for PCR were: 94°C, 1 min, 40°C annealing temperature for 1 min, and 72°C extension temperature for 1 min. These initial conditions were used for 4 cycles. Subsequently, the annealing temperature was raised to 50°C for additional 30 cycles. A 450 nucleotide fragment was observed and used as a probe to screen a human tonsilar B cell library. Positive clones were sequenced using standard dideoxynucleotide sequencing.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Washington University

(ii) TITLE OF INVENTION: BLNK PROTEINS

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Flehr, Hohbach, Test, Albritton & Herbert
(B) STREET: Four Embarcadero Center, Suite 3400
(C) CITY: San Francisco
(D) STATE: California
(E) COUNTRY: United States
(F) ZIP: 94111-4187

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US98/
(B) FILING DATE: Herewith
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/819,013
(B) FILING DATE: 17-MAR-1997

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/788,322
(B) FILING DATE: 24-JAN-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Silva, Robin M.
(B) REGISTRATION NUMBER: 38,304
(C) REFERENCE/DOCKET NUMBER: FP-64383-2/RFT/RMS

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (415) 781-1989
(B) TELEFAX: (415) 398-3249
(C) TELEX: 910 277299

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 456 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Asp Lys Leu Asn Lys Ile Thr Val Pro Ala Ser Gln Lys Leu Arg
1 5 10 15

Gln Leu Gln Lys Met Val His Asp Ile Lys Asn Asn Glu Gly Gly Ile
20 25 30

Met Asn Lys Ile Lys Lys Leu Lys Val Lys Ala Pro Pro Ser Val Pro
35 40 45

Arg Arg Asp Tyr Ala Ser Glu Ser Pro Ala Asp Glu Glu Glu Gln Trp
50 55 60

Ser Asp Asp Phe Asp Ser Asp Tyr Glu Asn Pro Asp Glu His Ser Asp
65 70 75 80

Ser Glu Met Tyr Val Met Pro Ala Glu Glu Asn Ala Asp Asp Ser Tyr
85 90 95

Glu Pro Pro Pro Val Glu Gln Glu Thr Arg Pro Val His Pro Ala Leu
100 105 110

Pro Phe Ala Arg Gly Glu Tyr Ile Asp Asn Arg Ser Ser Gln Arg His
115 120 125

Ser Pro Pro Phe Ser Lys Thr Leu Pro Ser Lys Pro Ser Trp Pro Ser
130 135 140

Glu Lys Ala Arg Leu Thr Ser Thr Leu Pro Ala Leu Thr Ala Leu Gln
145 150 155 160

Lys Pro Gln Val Pro Pro Lys Pro Lys Gly Leu Leu Glu Asp Glu Ala
165 170 175

Asp Tyr Val Val Pro Val Glu Asp Asn Asp Glu Asn Tyr Ile His Pro
180 185 190

Thr Glu Ser Ser Ser Pro Pro Pro Glu Lys Ala Pro Met Val Asn Arg
195 200 205

Ser Thr Lys Pro Asn Ser Ser Thr Pro Ala Ser Pro Pro Gly Thr Ala
210 215 220

Ser Gly Arg Asn Ser Gly Ala Trp Glu Thr Lys Ser Pro Pro Pro Ala
225 230 235 240

Ala Pro Ser Pro Leu Pro Arg Ala Gly Lys Lys Pro Thr Thr Pro Leu
245 250 255

Lys Thr Thr Pro Val Ala Ser Gln Gln Asn Ala Ser Ser Val Cys Glu
260 265 270

Glu Lys Pro Ile Pro Ala Glu Arg His Arg Gly Ser Ser His Arg Gln
275 280 285

Glu Ala Val Gln Ser Pro Val Phe Pro Pro Ala Gln Lys Gln Ile His
290 295 300

Gln Lys Pro Ile Pro Leu Pro Arg Phe Thr Glu Gly Gly Asn Pro Thr
305 310 315 320

Val Asp Gly Pro Leu Pro Ile Phe Ser Ser Asn Ser Thr Ile Ser Glu
325 330 335

Gln Glu Ala Gly Val Leu Cys Lys Pro Trp Tyr Ala Gly Ala Cys Asp
340 345 350

Arg Lys Ser Ala Glu Glu Ala Leu His Arg Ser Asn Lys Asp Gly Ser
355 360 365

Phe Leu Ile Arg Lys Ser Ser Gly His Asp Ser Lys Gln Pro Tyr Thr
370 375 380

Leu Val Val Phe Phe Asn Lys Arg Val Tyr Asn Ile Pro Val Arg Phe
385 390 395 400

Ile Glu Ala Thr Lys Gln Tyr Ala Leu Gly Arg Lys Lys Asn Gly Glu
405 410 415

Glu Tyr Phe Gly Ser Val Ala Glu Ile Ile Arg Asn His Gln His Ser
420 425 430

Pro Leu Val Leu Ile Asp Ser Gln Asn Asn Thr Lys Asp Ser Thr Arg
435 440 445

Leu Lys Tyr Ala Val Lys Val Ser
450 455

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1806 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCTTCGTGGC CGCAGCCTGC ACTCTCAGAA ATCAGACTTG AGTGGCCGGA ACCCTTGAGA 60

CCAGAGGCTT ACCATGCTGC TCCCTAGGAG GGCCAGGAAC TGCTGACGTG ACCACTGGAC 120

AGTTATTCGT GTCTCTTACA ATTACCAAAC AGAATGGACA AGCTTAATAA AATAACCGTC 180

CCCGCCAGTC AGAAGTTGAG GCAGCTTCAA AAGATGGTCC ATGATATTAA AAACAATGAA 240

GGTGGAATAA TGAATAAAAT CAAAAAGCTA AAAGTCAAAG CACCTCCAAG TGTTCCTCGA 300

AGGGACTACG CTTCAGAGAG CCCCGCTGAC GAAGAGGAGC AGTGGTCCGA TGACTTTGAC 360

AGCGACTATG AAAATCCAGA TGAGCACTCG GACTCAGAGA TGTACGTGAT GCCCGCCGAG 420

GAGAACGCTG ATGACAGCTA CGAGCCGCCT CCAGTAGAGC AGGAAACCAG GCCGGTTCAC 480

CCAGCCCTGC CCTTCGCCAG AGGCGAGTAT ATAGACAATC GATCAAGCCA GAGGCATTCC 540

CCACCCTTCA GCAAGACACT TCCCAGTAAG CCCAGCTGGC CTTCAGAGAA AGCAAGGCTC 600

ACCTCCACCC TGCCGGCCCT GACTGCTTTG CAGAAACCTC AAGTCCCACC CAAACCCAAA 660

GGCCTCCTTG AGGATGAGGC TGATTATGTG GTCCCCGTGG AAGATAATGA TGAAAACTAT 720

ATTCATCCCA CAGAAAGCAG TTCACCTCCA CCTGAAAAAG CTCCCATGGT GAATAGATCA 780

ACCAAGCCAA ATTCCTCAAC GCCCGCCTCT CCTCCAGGAA CAGCTTCAGG TCGAAACAGT 840

GGGGCCTGGG AAACCAAGTC ACCTCCACCA GCTGCACCAT CCCCGTTGCC ACGGGCCGGG 900

AAAAAACCAA CGACACCACT GAAGACAACT CCAGTTGCCT CTCAACAGAA TGCTTCAAGT 960

GTTTGTGAAG AAAAACCTAT ACCTGCTGAA CGCCACCGAG GGTCAAGTCA CAGACAAGAA 1020

GCTGTGCAGT CACCAGTGTT TCCTCCTGCC CAGAAACAAA TCCACCAAAA ACCCATACCT 1080

CTGCCAAGAT TTACAGAAGG GGGAAACCCA ACTGTGGATG GGCCCCTACC CAGCTTTTCA 1140

TCTAATTCCA CTATTTCAGA ACAGGAAGCT GGCGTTCTCT GCAAGCCATG GTATGCTGGA 1200

GCCTGTGATC GAAAGTCTGC TGAAGAGGCA TTGCACAGAT CAAACAAGGA TGGATCATTT 1260

CTTATTCGGA AAAGCTCTGG CCATGATTCC AAACAACCAT ATACACTAGT TGTATTCTTT 1320

AATAAGCGAG TATATAATAT TCCTGTGCGA TTTATTGAAG CAACAAAACA ATATGCCTTG 1380

GGCAGAAAGA AAAATGGTGA AGAGTACTTT GGAAGTGTTG CTGAAATCAT CAGGAATCAT 1440

CAACATAGTC CTTTGGTTCT TATTGACAGT CAGAATAACA CAAAAGATTC CACCAGACTG 1500

AAGTATGCAG TTAAAGTTTC ATAAAGGGGG AAAAAAAAGA TCAATACCAT TGCTTCAGAC 1560

ACTTTCCCAA AGTTTCTCCT TTTGAGAAAA AGTCCCAAAA CTTCATATTT TGGATTATGA 1620

ATCATCCAGT AATAAAATGG AAGATGGAGT CAGCTATTGA AGTGGTCATC CATTTCTTTT 1680

TAAGAAGCTC ATGTGGACTT GTTCTATTGC CTGACCTGAT GAACTGTTAA TATCTGGTGA 1740

GGTTGAGTTA TCATGCTACT AATATTTTCC AAATAAATAT TTTTATTTTT AAAAAAAAAA 1800

AAAAAA 1806

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 449 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Asp Lys Leu Asn Lys Ile Thr Val Pro Ala Ser Gln Lys Leu Arg
1 5 10 15

His Ile Lys Asn Asn Glu Gly Gly Ile Met Asn Lys Ile Lys Lys Leu
20 25 30

Lys Val Lys Ala Pro Pro Ser Val Pro Arg Arg Asp Tyr Ala Ser Glu
35 40 45

Ser Pro Ala Asp Glu Glu Glu Gln Trp Ser Asp Asp Phe Asp Ser Asp
50 55 60

Tyr Glu Asn Pro Asp Glu His Ser Asp Ser Glu Met Tyr Val Met Pro
65 70 75 80

Ala Glu Glu Asn Ala Asp Asp Ser Tyr Glu Pro Pro Pro Val Glu Gln
85 90 95

Glu Thr Arg Pro Val His Pro Ala Leu Pro Phe Ala Arg Gly Glu Tyr
100 105 110

Ile Asp Asn Arg Ser Ser Gln Arg His Ser Pro Pro Phe Ser Lys Thr
115 120 125

Leu Pro Ser Lys Pro Ser Trp Pro Ser Glu Lys Ala Arg Leu Thr Ser
130 135 140

Thr Leu Pro Ala Leu Thr Ala Leu Gln Lys Pro Gln Val Pro Pro Lys
145 150 155 160

Pro Lys Gly Leu Leu Glu Asp Glu Ala Asp Tyr Val Val Pro Val Glu
165 170 175

Asp Asn Asp Glu Asn Tyr Ile His Pro Thr Glu Ser Ser Ser Pro Pro
180 185 190

Pro Glu Lys Ala Pro Met Val Asn Arg Ser Thr Lys Pro Asn Ser Ser
195 200 205

Thr Pro Ala Ser Pro Pro Gly Thr Ala Ser Gly Arg Asn Ser Gly Ala
210 215 220

Trp Glu Thr Lys Ser Pro Pro Pro Ala Ala Pro Ser Pro Leu Pro Arg
225 230 235 240

Ala Gly Lys Lys Pro Thr Thr Pro Leu Lys Thr Thr Pro Val Ala Ser
245 250 255

Gln Gln Asn Ala Ser Ser Val Cys Glu Glu Lys Pro Ile Pro Ala Glu
260 265 270

Arg His Arg Gly Ser Ser His Arg Gln Glu Ala Val Gln Ser Pro Val
275 280 285

Phe Pro Pro Ala Gln Lys Gln Ile His Gln Lys Pro Ile Pro Leu Pro
290 295 300

Arg Phe Thr Glu Gly Gly Asn Pro Thr Val Asp Gly Pro Leu Pro Ser
305 310 315 320

Phe Ser Ser Asn Ser Thr Ile Ser Glu Gln Glu Ala Gly Val Leu Cys
325 330 335

Lys Pro Trp Tyr Ala Gly Ala Cys Asp Arg Lys Ser Ala Glu Glu Ala
340 345 350

Leu His Arg Ser Asn Lys Asp Gly Ser Phe Leu Ile Arg Lys Ser Ser
355 360 365

Gly His Asp Ser Lys Gln Pro Tyr Thr Leu Val Val Phe Phe Asn Lys
370 375 380

Arg Val Tyr Asn Ile Pro Val Arg Phe Ile Glu Ala Thr Lys Gln Tyr
385 390 395 400

Ala Leu Gly Arg Lys Lys Asn Gly Glu Glu Tyr Phe Gly Ser Val Ala
405 410 415

Glu Ile Ile Arg Asn His Gln His Ser Pro Leu Val Leu Ile Asp Ser
420 425 430

Gln Asn Asn Thr Lys Asp Ser Thr Arg Leu Lys Tyr Ala Val Lys Val
435 440 445

Ser

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1785 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCTTCGTGGC CGCAGCCTGC ACTCTCAGAA ATCAGACTTG AGTGGCCGGA ACCCTTGAGA 60

CCAGAGGCTT ACCATGCTGC TCCCTAGGAG GGCCAGGAAC TGCTGACGTG ACCACTGGAC 120

AGTTATTCGT GTCTCTTACA ATTACCAAAC AGAATGGACA AGCTTAATAA AATAACCGTC 180

CCCGCCAGTC AGAAGTTGAG GCATATTAAA AACAATGAAG GTGGAATAAT GAATAAAATC 240

AAAAAGCTAA AAGTCAAAGC ACCTCCAAGT GTTCCTCGAA GGGACTACGC TTCAGAGAGC 300

CCCGCTGACG AAGAGGAGCA GTGGTCCGAT GACTTTGACA GCGACTATGA AAATCCAGAT 360

GAGCACTCGG ACTCAGAGAT GTACGTGATG CCCGCCGAGG AGAACGCTGA TGACAGCTAC 420

GAGCCGCCTC CAGTAGAGCA GGAAACCAGG CCGGTTCACC CAGCCCTGCC CTTCGCCAGA 480

GGCGAGTATA TAGACAATCG ATCAAGCCAG AGGCATTCCC CACCCTTCAG CAAGACACTT 540

CCCAGTAAGC CCAGCTGGCC TTCAGAGAAA GCAAGGCTCA CCTCCACCCT GCCGGCCCTG 600

ACTGCTTTGC AGAAACCTCA AGTCCCACCC AAACCCAAAG GCCTCCTTGA GGATGAGGCT 660

GATTATGTGG TCCCCGTGGA AGATAATGAT GAAAACTATA TTCATCCCAC AGAAAGCAGT 720

TCACCTCCAC CTGAAAAAGC TCCCATGGTG AATAGATCAA CCAAGCCAAA TTCCTCAACG 780

CCCGCCTCTC CTCCAGGAAC AGCTTCAGGT CGAAACAGTG GGGCCTGGGA AACCAAGTCA 840

CCTCCACCAG CTGCACCATC CCCGTTGCCA CGGGCCGGGA AAAAACCAAC GACACCACTG 900

AAGACAACTC CAGTTGCCTC TCAACAGAAT GCTTCAAGTG TTTGTGAAGA AAAACCTATA 960

CCTGCTGAAC GCCACCGAGG GTCAAGTCAC AGACAAGAAG CTGTGCAGTC ACCAGTGTTT 1020

CCTCCTGCCC AGAAACAAAT CCACCAAAAA CCCATACCTC TGCCAAGATT TACAGAAGGG 1080

GGAAACCCAA CTGTGGATGG GCCCCTACCC AGCTTTTCAT CTAATTCCAC TATTTCAGAA 1140

CAGGAAGCTG GCGTTCTCTG CAAGCCATGG TATGCTGGAG CCTGTGATCG AAAGTCTGCT 1200

GAAGAGGCAT TGCACAGATC AAACAAGGAT GGATCATTTC TTATTCGGAA AAGCTCTGGC 1260

CATGATTCCA AACAACCATA TACACTAGTT GTATTCTTTA ATAAGCGAGT ATATAATATT 1320

CCTGTGCGAT TTATTGAAGC AACAAAACAA TATGCCTTGG GCAGAAAGAA AAATGGTGAA 1380

GAGTACTTTG GAAGTGTTGC TGAAATCATC AGGAATCATC AACATAGTCC TTTGGTTCTT 1440

ATTGACAGTC AGAATAACAC AAAAGATTCC ACCAGACTGA AGTATGCAGT TAAAGTTTCA 1500

TAAAGGGGGA AAAAAAAGAT CAATACCATT GCTTCAGACA CTTTCCCAAA GTTTCTCCTT 1560

TTGAGAAAAA GTCCCAAAAC TTCATATTTT GGATTATGAA TCATCCAGTA ATAAAATGGA 1620

AGATGGAGTC AGCTATTGAA GTGGTCATCC ATTTCTTTTT AAGAAGCTCA TGTGGACTTG 1680

TTCTATTGCC TGACCTGATG AACTGTTAAT ATCTGGTGAG GTTGAGTTAT CATGCTACTA 1740

ATATTTTCCA AATAAATATT TTTATTTTTA AAAAAAAAAA AAAAA 1785

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 457 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Asp Lys Leu Asn Lys Ile Thr Val Pro Ala Ser Gln Lys Leu Arg
1 5 10 15

Gln Leu Gln Lys Met Val His Asp Ile Lys Asn Asn Glu Gly Gly Ile
20 25 30

Met Asp Lys Ile Lys Lys Leu Lys Val Lys Gly Pro Pro Ser Val Pro
35 40 45

Arg Arg Asp Tyr Ala Leu Asp Ser Pro Ala Asp Glu Glu Glu Gln Trp
50 55 60

Ser Asp Asp Phe Asp Ser Asp Tyr Glu Asn Pro Asp Glu His Ser Asp
65 70 75 80

Ser Glu Met Tyr Val Met Pro Ala Glu Glu Thr Gly Asp Asp Ser Tyr
85 90 95

Glu Pro Pro Pro Ala Glu Gln Gln Thr Arg Val Val His Pro Ala Leu
100 105 110

Pro Phe Thr Arg Gly Glu Tyr Val Asp Asn Arg Ser Ser Gln Arg His
115 120 125

Ser Pro Pro Phe Ser Lys Thr Leu Pro Ser Lys Pro Ser Trp Pro Ser
130 135 140

Ala Lys Ala Arg Leu Ala Ser Thr Leu Pro Ala Pro Asn Ser Leu Gln
145 150 155 160

Lys Pro Gln Val Pro Pro Lys Pro Lys Asp Leu Leu Glu Asp Glu Ala
165 170 175

Asp Tyr Val Val Pro Val Glu Asp Asn Asp Glu Asn Tyr Ile His Pro
180 185 190

Arg Glu Ser Ser Pro Pro Pro Ala Glu Lys Ala Pro Met Val Asn Arg
195 200 205

Ser Thr Lys Pro Asn Ser Ser Ser Lys His Met Ser Pro Pro Gly Thr
210 215 220

Val Ala Gly Arg Asn Ser Gly Val Trp Asp Ser Lys Ser Ser Leu Pro
225 230 235 240

Ala Ala Pro Ser Pro Leu Pro Arg Ala Gly Lys Lys Pro Ala Thr Pro
245 250 255

Leu Lys Thr Thr Pro Val Pro Pro Leu Pro Asn Ala Ser Asn Val Cys
260 265 270

Glu Glu Lys Pro Val Pro Ala Glu Arg His Arg Gly Ser Ser His Arg
275 280 285

Gln Asp Thr Val Gln Ser Pro Val Phe Pro Pro Thr Gln Lys Pro Val
290 295 300

His Gln Lys Pro Val Pro Leu Pro Arg Phe Pro Glu Ala Gly Ser Pro
305 310 315 320

Ala Ala Asp Gly Pro Phe His Ser Phe Pro Phe Asn Leu Thr Phe Ala
325 330 335

Asp Gln Glu Gly Glu Leu Leu Gly Lys Pro Trp Tyr Ala Gly Ala Cys
340 345 350

Asp Arg Lys Phe Ala Glu Glu Ala Leu His Arg Ser Asn Lys Asp Gly
355 360 365

Ser Phe Leu Ile Arg Lys Ser Phe Gly His Asp Ser Lys Gln Pro Tyr
370 375 380

Thr Leu Val Ala Phe Phe Asn Lys Arg Val Tyr Asn Ile Pro Val Arg
385 390 395 400

Phe Ile Glu Ala Thr Lys Gln Tyr Ala Leu Gly Lys Lys Lys Asn Gly
405 410 415

Glu Glu Tyr Phe Gly Ser Val Val Glu Ile Val Asn Ser His Gln His
420 425 430

Asn Pro Leu Val Leu Ile Asp Ser Gln Asn Asn Thr Lys Asp Ser Thr
435 440 445

Arg Leu Lys Tyr Ala Val Lys Val Ser
450 455

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1718 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTGTGGGTTG CTCGCAGAAG TCAGTTCCAG TGGCTTGAGT TCTTGAGGCC AGAGCCTTAC 60

CATGCTGCTC CCCAGGAAGT CCAGGAGCTG CTGACACCCC CCTGGACAGC GACACATCCT 120

CTCTCAAGAA AATGGACAAG CTGAATAAGA TAACTGTCCC TGCCAGCCAG AAGCTGAGAC 180

AGCTTCAAAA GATGGTCCAT GATATTAAGA ACAATGAAGG TGGAATAATG GACAAGATAA 240

AAAAGCTAAA AGTCAAAGGC CCTCCAAGTG TTCCTCGAAG GGACTATGCA TTAGACAGCC 300

CTGCAGATGA AGAGGAGCAG TGGTCAGATG ACTTCGACAG TGACTATGAA AATCCAGATG 360

AACATTCGGA CTCCGAGATG TATGTGATGC CTGCCGAGGA GACGGGCGAC GATTCCTATG 420

AACCGCCTCC CGCTGAGCAG CAGACACGGG TGGTCCATCC AGCCCTGCCC TTCACGAGGG 480

GCGAGTATGT AGATAATCGA TCCAGCCAGC GGCACTCTCC GCCCTTCAGC AAGACACTTC 540

CCAGTAAGCC CAGCTGGCCT TCAGCGAAAG CGAGGCTGGC CTCCACTCTG CCAGCCCCCA 600

ACTCTCTACA GAAGCCTCAA GTCCCCCCCA AGCCCAAAGA CCTCCTTGAG GATGAGGCTG 660

ATTATGTGGT CCCTGTGGAA GATAACGATG AAAACTATAT CCATCCCAGA GAAAGTAGCC 720

CGCCGCCTGC TGAGAAGGCT CCCATGGTGA ATAGATCAAC CAAGCCAAAC AGTTCCTCAA 780

AGCACATGTC GCCTCCAGGG ACTGTCGCAG GTCGAAACAG TGGGGTCTGG GACTCCAAGT 840

CATCTTTGCC TGCCGCACCA TCCCCACTAC CACGGGCTGG GAAGAAGCCA GCTACACCAC 900

TTAAGACTAC TCCCGTTCCT CCCCTACCGA ATGCATCAAA TGTTTGTGAA GAAAAGCCTG 960

TTCCTGCTGA GCGCCACCGA GGGTCTAGTC ACAGACAAGA CACTGTACAG TCACCAGTGT 1020

TTCCTCCCAC CCAGAAACCT GTCCATCAAA AGCCTGTACC CTTGCCAAGG TTCCCAGAAG 1080

CGGGGAGCCC AGCTGCAGAT GGACCGTTCC ACAGCTTCCC ATTTAATTTG ACGTTTGCAG 1140

ACCAGGAGGG TGAACTGCTC GGTAAGCCCT GGTATGCTGG CGCCTGTGAC CGCAAGTTTG 1200

CTGAAGAGGC CTTGCACAGA TCCAACAAGG ATGGATCGTT TCTTATTCGG AAGAGCTTTG 1260

GCCATGATTC CAAGCAGCCG TACACCCTAG TTGCGTTCTT TAACAAGCGA GTGTATAATA 1320

TTCCTGTACG GTTTATTGAA GCAACCAAAC AGTATGCTTT GGGAAAGAAG AAAAATGGTG 1380

AAGAGTACTT CGGAAGTGTT GTGGAAATCG TCAACAGTCA TCAGCACAAC CCCCTGGTTC 1440

TTATTGACAG TCAGAATAAC ACGAAAGATT CCACGAGACT GAAATATGCT GTGAAGGTTT 1500

CATAACGATA CCACGGTTCC AGACATGTCC TCTGTTTCTT CTTTTGAGAA AACATCATAT 1560

TCTGGCTATG ACTCCTCAGC AGTAAGAGAG AAAAGATGAA TGAAGCCACT GAGGCTTCGT 1620

GAATGAATGA ATCTACTCCT TCCTAGGGCG TTCACACGAG CTTTTCTATC ACCTGACCTG 1680

ACGAAGTCAT AGCTGGGGAG GTTCGGTTAC TATGATAC 1718

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Tyr Glu Xaa Pro
1

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1..2
(D) OTHER INFORMATION: /note= "The 'X' appearing at positions 1, 8 and 15, represent either Aspartic Acid or Glutamic Acid."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Xaa Xaa Tyr Glu Xaa Pro Xaa Xaa Xaa Tyr Xaa Xaa Pro Xaa Xaa Xaa
1 5 10 15

Tyr Glu Pro Pro
20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 19..20

(D) OTHER INFORMATION: /note= "The 'N' at position 19 represents Inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCGAGAATTC AARAARCCNA CNACNCC 27

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Lys Pro Thr Thr Pro Leu Lys
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 13..14
(D) OTHER INFORMATION: /note= "The 'N' at position 13 represents Inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTGAGGATCC TTNGTNGCYT CDATRAA 27

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Lys Arg Val Tyr Asn Ile Pro Val Arg Phe Ile Glu Ala Thr Lys
1 5 10 15

CLAIMS

We claim:

1. A recombinant nucleic acid encoding a BLNK protein.

2. A recombinant nucleic acid according to claim 1 that is at least 60% identical to the sequence depicted in Figure 2.

3. A recombinant nucleic acid according to claim 1 wherein said BLNK protein is a human BLNK protein.

4. A recombinant nucleic acid according to claim 1 encoding the amino acid sequence depicted in Figure 1.

5. A recombinant nucleic acid according to claim 1 which will hybridize to the nucleic acid depicted in Figure 2 under high stringency conditions.

6. A recombinant nucleic acid according to claim 1 comprising the nucleic acid depicted in Figure 2.

7. An expression vector comprising transcriptional and translational regulatory DNA operably linked to DNA encoding a BLNK protein.

8. A host cell transformed with the nucleic acid of claim 1.

9. A host cell transformed with an expression vector according to claim 7.

10. A method of producing a BLNK protein comprising:

a) culturing a host cell transformed with nucleic acid encoding a BLNK protein; and

b) expressing said nucleic acid to produce a BLNK protein.

11. A recombinant BLNK protein.

12. A recombinant BLNK protein according to claim 11 encoded by a nucleic acid which hybridizes to the nucleic acid sequence shown in Figure 2 under high stringency conditions.

13. A recombinant BLNK protein according to claim 11 encoded by a nucleic acid which is at least 60% identical to the nucleic acid sequence shown in Figure 2.

14. A recombinant BLNK protein according to claim 11 which is at least about 50% homologous to the amino acid sequence shown in Figure 1.

15. A recombinant BLNK protein according to claim 11 which has the amino acid sequence shown in Figure 1.

16. A pharmaceutical composition comprising a BLNK protein.

17. A polypeptide capable of specifically binding to a BLNK 1 protein.

18. A polypeptide according to claim 17 wherein said polypeptide is an antibody.

19. An antibody which binds a BLNK protein.

20. A method for detecting a BLNK protein in a target sample comprising contacting a labelled polypeptide according to claim 17 with said target sample and assaying for the presence of binding between said labelled polypeptide and BLNK, if present, in said target sample.

21. A method for screening for a bioactive agent capable of binding to a BLNK protein, said method comprising combining a BLNK protein and a candidate bioactive agent, and determining the binding of said candidate agent to BLNK protein.

22. A method for screening for a bioactive agent capable of modulating the bioactivity of a BLNK protein, said method comprising the steps of:

a) combining:

i) a BLNK protein;

ii) a candidate bioactive agent; and

iii) a protein selected from the group consisting of Grb2 and PLC-γ; and

b) determining the binding of said protein to said BLNK protein;

wherein the absence of binding of said protein to said BLNK protein indicates that said agent is capable of modulating the bioactivity of said BLNK protein.

)KLNKITVPASQKLRQLQKMVHDIKNNEGGIMNKIKKLKVKAPPSVPRRDYASESPADEEEQ
IDDFDSDYENPDEHSDSEMYVMPAEENADDSYEPPPVEQETRPVHPALPFARGEYIDNRSSQ
ISPPFSKTLPSKPSWPSEKARLTSTLPALTALQKPQVPPKPKGLLEDEADYVVPVEDNDENY
IPTESSSPPPEKAPMVNRSTKPNSSTPASPPGTASGRNSGAWETKSPPPAAPSPLPRAGKKP
'PLKTTPVASQQNASSVCEEKPIPAERHRGSSHRQEAVQSPVFPPAQKQIHQKPIPLPRFTE
INPTVDGPLPIFSSNSTISEQEAGVLCKPWYAGACDRKSAEEALHRSNKDGSFLIRKSSGHD
:QPYTLVVFFNKRVYNIPVRFIEATKQYALGRKKNGEEYFGSVAEIIRNHQHSPLVLIDSQN
!KDSTRLKYAVKVS*

FIG._1

)KLNKITVPASQKLRQLQKMVHDIKNNEGGIMDKIKKLKVKGPPSVPRRDYALDSPAD
IEQWSDDFDSDYENPDEHSDSEMYVMPAEETGDDSYEPPPAEQQTRVVHPALPFTRGE
'DNRSSQRHSPPFSKTLPSKPSWPSAKARLASTLPAPNSLQKPQVPPKPKDLLEDEAD
'VPVEDNDENYIHPRESSPPPAEKAPMVNRSTKPNSSSKHMSPPGTVAGRNSGVWDSK
ILPAAPSPLPRAGKKPATPLKTTPVPPLPNASNVCEEKPVPAERHRGSSHRQDTVQSP
'PPTQKPVHQKPVPLPRFPEAGSPAADGPFHSFPFNLTFADQEGELLGKPWYAGACDR
'AEEALHRSNKDGSFLIRKSFGHDSKQPYTLVAFFNKRVYNIPVRFIEATKQYALGKK
IGEEYFGSVVEIVNSHQHNPLVLIDSQNNTKDSTRLKYAVKVS

FIG._5

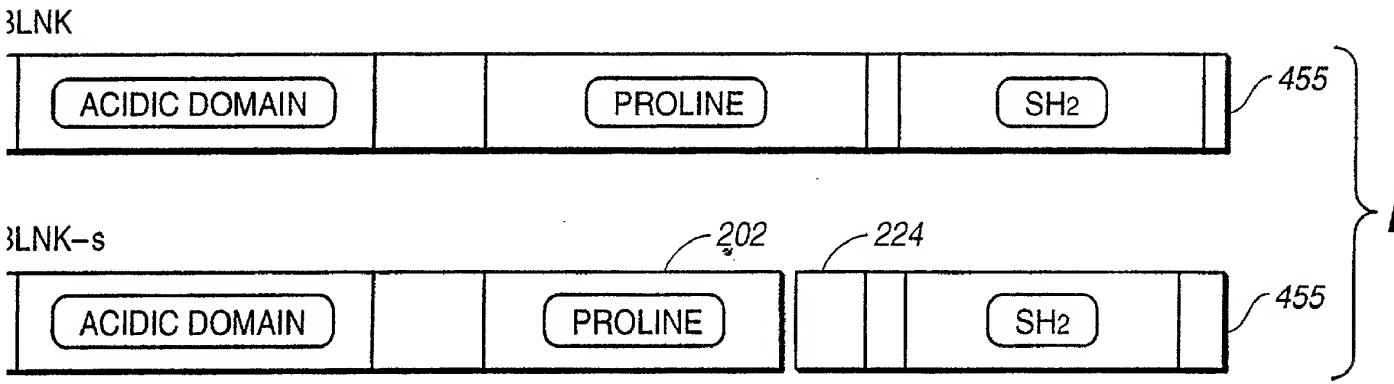

FIG._7

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CCTTCGTGGCCGCAGCCTGCACTCTCAGAAATCAGACTTGAGTGGCCGGAACCCTTGAGACCA
GAGGCTTACCATGCTGCTCCCTAGGAGGGCCAGGAACTGCTGACGTGACCACACTGGACAGTTAT
TCGTGTCTCTTACAATTACCAAACAGAATGGACAAGCTTAATAAATAACCGTCCCCCGCCAGT
CAGAAGTTGAGGCAGCTTCAAAAGATGGTCCATGATATTAAAAAACAAATGAAGGTGGAATAATG
AATAAAATCAAAAAGCTAAAAGTCAAAGCACCTCCAAGTGTTCCTCGAAGGGACTACGCTTCA
GAGAGCCCCGCTGACGAAGAGGAGCAGTGGTCCGATGACTTTGACAGCGACTATGAAAATCCA
GATGAGCACACTCGGACTCAGAGATGTACGTGATGCCCGCCGAGGAGAACGCTGATGACAGCTAC
GAGCCGCCTCCAGTAGAGCAGGAAACCAGGCCGGTTCACACCCAGCCCCTGCCCCTTCGCCAGAGGC
GAGTATATAGACAATCGATCAAGCCAGAGGCATTCCCCCACACCCTTCAGCAAGACACACTTCCCAGT
AAGCCCCAGCTGGCCTTCAGAGAAAGCAAGGCTCACACCCTCCACACCCTGCCGGCCCCTGACTGCTTTG
CAGAAACCCTCAAGTCCCACACCCAAACCCCAAAGGCCTCCCTTGAGGATGAGGCTGATTATGTGGTC
CCCGTGGAAGATAATGATGAAAACTATATTCATCCCACAGAAAGCAGTTCACCCTCCCACCCTGAA
AAAGCTCCCATGGTGAATAGATCAACCAAGCCAAAATTCCTCAACGCCCGCCCTCTCCTCCAGGA
ACAGCTTCAGGTCGAAACACAGTGGGGCCTGGGAAACCCAAGTCCACCCTCCCAGCTGCACCCATCC
CCGTTGCCACGGGCCGGAAAAAACCAACGACACCACTGAAGACAACTCCCAGTTGCCCTCTCAA
CAGAATGCTTCAAGTGTTTGTGAAGAAAAACCCTATACCTGCTGAACGCCCACCGAGGGTCAAGT
CACAGACAAGAAGCTGTGCAGTCACCCAGTGTTTCCCTCCTGCCCCAGAAACAAATCCCACCCAAAAA
CCCATACCTCCTGCCAAGAAGTTTACAGAAGGGGGAAACCCCAACTGTGGATGGGCCCCCTACCCCAGC
TTTTCATCTCTAATTCCCACTATTTCAGAACAGGAAGCTGGCGTTCTCTGCAAGCCCATGGTATGCCT
GGAGCCCTGTGATCGAAAGTCTGCTGAAGAGGCATTGCACACAGATCAAACAAGGATGGATCATTTT
CTTATTCGGAAAAAGCTCTGGCCATGATTCCCAAACAACCATATACACTAGTTGTATTCTTTTAAT
AAGCGAGTATATAATATTCCTGTGCGATTTATTGAAGCAACAAAACAATATGCCCTTGGGCAGA
AAGAAAAATGGTGAAGAGTACTTTGGAAGTGTTGCTGAAAATCATCAGGAATCATCAACATAGT
CCTTTGGTTCTTATTGACAGTCAGAATAACACAAAAGATTCCCACCAGACTGAAGTATGCAGTT
AAAGTTTCATAAAGGGGGAAAAAAAAGATCAATACCATTGCTTCAGACACACTTTCCCAAAGTTT
CTCCTTTTGAGAAAAAGTCCCAAAACTTCATATTTTGGATTATGAATCATCCAGTAATAAAAT
GGAAGATGGAGTCAGCTATTGAAGTGGTCATCCCATTTCTTTTTAAGAAGCTCATGTGGACTTG
TTCTATTGCCTGACCTGATGAACTGTTAATATCTGGTGAGGTTGAGTTATCATGCTACTAATA
TTTTCCAAATAAATATTTTTATTTTTAAAAAAAAAAAAAAA
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FIG._2

Met Asp Lys Leu Asn Lys Ile Thr Val Pro Ala Ser Gln Lys Leu Arg
1 5 10 15

His Ile Lys Asn Asn Glu Gly Gly Ile Met Asn Lys Ile Lys Lys Leu
20 25 30

Lys Val Lys Ala Pro Pro Ser Val Pro Arg Arg Asp Tyr Ala Ser Glu
35 40 45

Ser Pro Ala Asp Glu Glu Glu Gln Trp Ser Asp Asp Phe Asp Ser Asp
50 55 60

Tyr Glu Asn Pro Asp Glu His Ser Asp Ser Glu Met Tyr Val Met Pro
65 70 75 80

Ala Glu Glu Asn Ala Asp Asp Ser Tyr Glu Pro Pro Pro Val Glu Gln
85 90 95

Glu Thr Arg Pro Val His Pro Ala Leu Pro Phe Ala Arg Gly Glu Tyr
100 105 110

Ile Asp Asn Arg Ser Ser Gln Arg His Ser Pro Pro Phe Ser Lys Thr
115 120 125

Leu Pro Ser Lys Pro Ser Trp Pro Ser Glu Lys Ala Arg Leu Thr Ser
130 135 140

Thr Leu Pro Ala Leu Thr Ala Leu Gln Lys Pro Gln Val Pro Pro Lys
145 150 155 160

Pro Lys Gly Leu Leu Glu Asp Glu Ala Asp Tyr Val Val Pro Val Glu
165 170 175

Asp Asn Asp Glu Asn Tyr Ile His Pro Thr Glu Ser Ser Ser Pro Pro
180 185 190

Pro Glu Lys Ala Pro Met Val Asn Arg Ser Thr Lys Pro Asn Ser Ser
195 200 205

Thr Pro Ala Ser Pro Pro Gly Thr Ala Ser Gly Arg Asn Ser Gly Ala
210 215 220

Trp Glu Thr Lys Ser Pro Pro Pro Ala Ala Pro Ser Pro Leu Pro Arg
225 230 235 240

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Ala Gly Lys Lys Pro Thr Thr Pro Leu Lys Thr Thr Pro Val Ala Ser
                245                 250                 255

Gln Gln Asn Ala Ser Ser Val Cys Glu Glu Lys Pro Ile Pro Ala Glu
            260                 265                 270

Arg His Arg Gly Ser Ser His Arg Gln Glu Ala Val Gln Ser Pro Val
        275                 280                 285

Phe Pro Pro Ala Gln Lys Gln Ile His Gln Lys Pro Ile Pro Leu Pro
    290                 295                 300

Arg Phe Thr Glu Gly Gly Asn Pro Thr Val Asp Gly Pro Leu Pro Ser
305                 310                 315                 320

Phe Ser Ser Asn Ser Thr Ile Ser Glu Gln Glu Ala Gly Val Leu Cys
                325                 330                 335

Lys Pro Trp Tyr Ala Gly Ala Cys Asp Arg Lys Ser Ala Glu Glu Ala
            340                 345                 350

Leu His Arg Ser Asn Lys Asp Gly Ser Phe Leu Ile Arg Lys Ser Ser
        355                 360                 365

Gly His Asp Ser Lys Gln Pro Tyr Thr Leu Val Val Phe Phe Asn Lys
    370                 375                 380

Arg Val Tyr Asn Ile Pro Val Arg Phe Ile Glu Ala Thr Lys Gln Tyr
385                 390                 395                 400

Ala Leu Gly Arg Lys Lys Asn Gly Glu Glu Tyr Phe Gly Ser Val Ala
                405                 410                 415

Glu Ile Ile Arg Asn His Gln His Ser Pro Leu Val Leu Ile Asp Ser
            420                 425                 430

Gln Asn Asn Thr Lys Asp Ser Thr Arg Leu Lys Tyr Ala Val Lys Val
        435                 440                 445

Ser
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FIG._3B

CCTTCGTGGC CGCAGCCTGC ACTCTCAGAA ATCAGACTTG AGTGGCCGGA ACCCTTGAGA 60

CCAGAGGCTT ACCATGCTGC TCCCTAGGAG GGCCAGGAAC TGCTGACGTG ACCACTGGAC 120

AGTTATTCGT GTCTCTTACA ATTACCAAAC AGAATGGACA AGCTTAATAA AATAACCGTC 180

CCCGCCAGTC AGAAGTTGAG GCATATTAAA AACAATGAAG GTGGAATAAT GAATAAAATC 240

AAAAAGCTAA AAGTCAAAGC ACCTCCAAGT GTTCCTCGAA GGGACTACGC TTCAGAGAGC 300

CCCGCTGACG AAGAGGAGCA GTGGTCCGAT GACTTTGACA GCGACTATGA AAATCCAGAT 360

GAGCACTCGG ACTCAGAGAT GTACGTGATG CCCGCCGAGG AGAACGCTGA TGACAGCTAC 420

GAGCCGCCTC CAGTAGAGCA GGAAACCAGG CCGGTTCACC CAGCCCTGCC CTTCGCCAGA 480

GGCGAGTATA TAGACAATCG ATCAAGCCAG AGGCATTCCC CACCCTTCAG CAAGACACTT 540

CCCAGTAAGC CCAGCTGGCC TTCAGAGAAA GCAAGGCTCA CCTCCACCCT GCCGGCCCTG 600

ACTGCTTTGC AGAAACCTCA AGTCCCACCC AAACCCAAAG GCCTCCTTGA GGATGAGGCT 660

GATTATGTGG TCCCCGTGGA AGATAATGAT GAAAACTATA TTCATCCCAC AGAAAGCAGT 720

TCACCTCCAC CTGAAAAAGC TCCCATGGTG AATAGATCAA CCAAGCCAAA TTCCTCAACG 780

CCCGCCTCTC CTCCAGGAAC AGCTTCAGGT CGAAACAGTG GGGCCTGGGA AACCAAGTCA 840

CCTCCACCAG CTGCACCATC CCCGTTGCCA CGGGCCGGGA AAAAACCAAC GACACCACTG 900

AAGACAACTC CAGTTGCCTC TCAACAGAAT GCTTCAAGTG TTTGTGAAGA AAAACCTATA 960

CCTGCTGAAC GCCACCGAGG GTCAAGTCAC AGACAAGAAG CTGTGCAGTC ACCAGTGTTT 1020

CCTCCTGCCC AGAAACAAAT CCACCAAAAA CCCATACCTC TGCCAAGATT TACAGAAGGG 1080

GGAAACCCAA CTGTGGATGG GCCCCTACCC AGCTTTTCAT CTAATTCCAC TATTTCAGAA 1140

CAGGAAGCTG GCGTTCTCTG CAAGCCATGG TATGCTGGAG CCTGTGATCG AAAGTCTGCT 1200

GAAGAGGCAT TGCACAGATC AAACAAGGAT GGATCATTTC TTATTCGGAA AAGCTCTGGC 1260

CATGATTCCA AACAACCATA TACACTAGTT GTATTCTTTA ATAAGCGAGT ATATAATATT 1320

CCTGTGCGAT TTATTGAAGC AACAAAACAA TATGCCTTGG GCAGAAAGAA AAATGGTGAA 1380

GAGTACTTTG GAAGTGTTGC TGAAATCATC AGGAATCATC AACATAGTCC TTTGGTTCTT 1440

ATTGACAGTC AGAATAACAC AAAAGATTCC ACCAGACTGA AGTATGCAGT TAAAGTTTCA 1500

TAAAGGGGGA AAAAAAAGAT CAATACCATT GCTTCAGACA CTTTCCCAAA GTTTCTCCTT 1560

TTGAGAAAAA GTCCCAAAAC TTCATATTTT GGATTATGAA TCATCCAGTA ATAAAATGGA 1620

AGATGGAGTC AGCTATTGAA GTGGTCATCC ATTTCTTTTT AAGAAGCTCA TGTGGACTTG 1680

TTCTATTGCC TGACCTGATG AACTGTTAAT ATCTGGTGAG GTTGAGTTAT CATGCTACTA 1740

ATATTTTCCA AATAAATATT TTTATTTTTA AAAAAAAAAA AAAAA 1785

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CTGTGGGTTGCTCGCAGAAGTCAGTTCCAGTGGCTTGAGTTCTTGAGGCCAGAGCCTT
ACCATGCTGCTCCCCAGGAAGTCCAGGAGCTGCTGACACCCCCCTGGACAGCGACACA
TCCTCTCTCAAGAAAAATGGACAAGCTGAATAAGATAACTGTCCCCTGCCAGCCCAGAAGC
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CAAGATAAAAAAGCTAAAAAGTCAAAGGCCCCTCCAAGTGTTCCTCGAAGGGACTATGCA
TTAGACAGCCCCTGCAGATGAAGAGGAGCAGTGGTCAGATGACTTCGACACAGTGACTATG
AAAATCCAGATGAACATTCGGACTCCGAGATGTATGTGATGCCTGCCGAGGAGACGGG
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CTGCCCCTTCACGAGGGGCGAGTATGTAGATAATCGATCCCAGCCAGCGGCACACTCTCCGC
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GACCTCCCTTGAGGATGAGGCTGATTATGTGGTCCCTGTGGAAGATAACGATGAAAACT
ATATCCCATCCCCAGAGAAAGTAGCCCCGCCCTGCTGAGAAGGCTCCCCATGGTGAATAG
ATCAACCAAGCCCAAACACAGTTCCTCCAAAGCACACATGTCGCCCTCCCAGGGACTGTCGTCGCAGGT
CGAAACAGTGGGGGTCTGGGACTCCCAAGTCATCTTTGCCCTGCCGCACCATCCCCCACTAC
CACGGGCTGGGGAAGAAGCCAGCTACACCACTTAAGACTACTCCCCGTTCCTCCCCCTACC
GAATGCATCAAATGTTTGTGAAGAAAAAGCCCTGTTCCCTGCTGAGCGCCCACCGAGGGTCT
AGTCACAGACAAGACACACTGTACAGTCACCCAGTGTTTCCTCCCCACCCCAGAAAACCCTGTCC
ATCAAAAGCCCTGTGTACCCCTTGCCCAAGAAGGTTCCCCAGAAGCGGGGGAGCCCCAGCTGCAGATGG
ACCGTTCCCACAGCTTCCCCATTTTAATTTGACGTTTGCAGACCAGGAGAGGGTGAACTGCTC
GGTAAGCCCCTGGTATGCTGGCGCCCTGTGACCGCAAGTTTGCTGAAGAGGCCTTGCACA
GATCCAACAAGGATGGATCGTTTCTTATTCGGAAGAGCTTTGGCCATGATTCCCAAGCA
GCCGTACACACCCCTAGTTGCGTTCTTTAACAAGCGAGTGTATAATATTCCTGTACGGTTT
ATTGAAGCAACCAAACAGTATGCTTTGGGAAAGAAGAAAAATGGTGAAGAGTACTTCG
GAAGTGTTGTGGAAATCGTCAACACAGTCATCAGCCACAACCCCCCTGGTTCTTATTGACAG
TCAGAATAACACGAAAGATTCCACGAGACTGAAATATGCTGTGAAGGTTTCATAACGA
TACCACGGTTCCAGACATGTCCTCTTCTTTTGAGAAAACATCATATTCTGGC
TATGACTCCTCAGCAGTAAGAGAGAAAAGATGAATGAAGCCACTGAGGCTTCGTGAAT
GAATGAATCTACTCCCTTCCTAGGGCGTTCACACGAGCTTTCTATCACCTGACCTGAC
GAAGTCATAGCTGGGGAGGTTCGGTTACTATGATAC
```

FIG._6

INTERNATIONAL SEARCH REPORT

Ir onal Application No
PCT/US 98/01394

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 A61K38/17 G01N33/58 //C12N15/62

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JACKMAN, J.K. ET AL.: "Molecular cloning of SLP-76, a 76-kDa tyrosine phosphoprotein associated with Grb2 in T cells." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 13, 31 March 1995, pages 7029-7032, XP002064174 cited in the application	1,3, 7-11,17, 19-21
Y	see abstract; figure 2	22
Y	WO 96 30332 A (US HEALTH) 3 October 1996 see page 14, line 31 - line 34; claim 26; examples 8C,9; table 1	22
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
6 May 1998	22/05/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Smalt, R

INTERNATIONAL SEARCH REPORT

onal Application No
PCT/US 98/01394

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication.where appropriate, of the relevant passages	Relevant to claim No.
X	FU, C. ET AL.: "Identification of novel Grb2 and PLC-gamma binding proteins in B cell receptor (BCR) function." THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, vol. 99, no. 1, part 2, 1 January 1997, page S469, Abstr. 1905 XP002064175 see abstract	11-15, 17-22
P,X	DATABASE EMBL - EMROD Entry MMAJ814, Acc.No. AJ222814, 12 December 1997 COHEN, P.L. ET AL.: "Mus musculus mRNA for B cell specific protein." XP002064177 see the whole document	1,2,5, 7-14
P,X	FU, C. ET AL.: "Identification of two phosphoproteins, pp70 and pp68, which interact with phospholipase C-gamma, Grb2, and Vav after B cell antigen activation." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 43, 24 October 1997, pages 27362-27368, XP002064176 see the whole document	11-15, 17-22
A	RICHARD S ET AL: "ASSOCIATION OF P62, A MULTIFUNCTIONAL SH2- AND SH3-DOMAIN-BINDING PROTEIN, WITH SRC FAMILY TYROSINE KINASES, GBR2, AND PHOSPHOLIPASE CGAMMY-1" MOLECULAR AND CELLULAR BIOLOGY, vol. 15, no. 1, 1 January 1995, pages 186-197, XP000562643 see the whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/01394

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9630332 A	03-10-96	US 5688992 A	18-11-97
		AU 5377696 A	16-10-96
		CA 2215827 A	03-10-96
		EP 0820433 A	28-01-98